Activity of some enzymes of the carbon metabolism and H^+-pumps in root cells of wheat genotypes exposed to salt stress

H.G. Babayev*, U.A. Gurbanova, N.M. Guliyev

Laboratory of Enzymology of Photosynthetic Carbon Assimilation, Institute of Molecular Biology and Biotechnologies, Azerbaijan National Academy of Sciences, 11 Izzat Nabiyev, Baku AZ1073, Azerbaijan
**For correspondence: babayev_hg@yahoo.co.uk*

The role of carbonic anhydrase (CA), NAD-malate dehydrogenase (NAD-MDH), ribulose-1,5-bisphosphate carboxylase (RBPC) and H^+-pumps in the formation of adaptive reactions in plant roots in response to salinity has been studied in the wheat genotypes (Barakatli 95, Garagylchyg 2 and Gyrmyzy bughda) with contrasting productivity and drought tolerance. Controversial issues concerning the localization of CA in the plant root system were clarified and possible physiological and biochemical functions were discussed. CA, NAD-MDH, RBPC and H^+-pumps were found to function in concert in root cells of the wheat genotypes thereby participating in the formation of tolerance against stress effects.

Keywords: *Wheat, root cells, salt stress, CA, NAD-MDH, RBPC, H^+-pumps, tolerance*

INTRODUCTION

Abiotic environmental factors are known to affect photosynthesis by changing metabolism and limiting CO_2 amount fixed in the catrboxylation center (Chaves, Oliveria, 2004; Covshoff, Hibberd, 2012; Pinheiro, Chaves, 2011).

Similar to drought, high salinity levels also decrease crop productivity (Ershov, 2006) and sometimes completely destroy plants (Gambarova, Asadova, 2010). According to the literature data, plants are less tolerant to drought and salinity during the initial periods of ontogenesis (Weiping et al., 2010).

It is known that biochemical reactions, which are the basis of metabolism, proceed enzymatically (Stitt et al., 2002; Stitt, Gibon, 2014). From this point of view, the study of the carbon and malate metabolism enzymes in the formation of regulation mechanisms of metabolic processes is of great importance (Eprintseva et al., 2011). Accumulation of CO_2 in plant tissues as a dibasic acid-malate and its decarboxylation is realized by the various isoforms of MDH. Being widely spread in plants these enzymes have a complex isoenzyme specificity. They participate in the creation of stress tolerance by changing their activity, isoform number and subcellular localization depending on the plant growth stages and stress duration. Being in a state of dynamic balance, enzymes of the malate dehydrogenase system in plants are able to react to environmental changes, demands of the organism and physiological state (Pinheiro et al., 1991).

Another factor forming the basis of adaptivity of MDH enzymes is their polyfunctionality. Thus, in mitochondria, NAD-MDH participates in the Krebs cycle, in cytosol and peroxisomes in the malate-aspartate shuttle and in glyoxysomes in the oxidation of fatty acids (Beeler et al., 2014; Selinski et al., 2015; Zhijian et al., 2015).

The study of the regulation mechanisms of the physiological and biochemical properties of carbon metabolism enzymes is especially important for clarifying the adaptation mechanisms to stress. Therefore, the research on the enzymes such as CA realizing diffusion of CO_2 through stomata and transportation to the carboxylation center of RBPC is of great importance (Aliyev, Guliyev, 1990; Guliev et al., 2003).

H^+ electrochemical gradient or proton motive force is created by transport ATP-ases of membrane or redox-pumps, performing the proton excretion function. This gradient can be expended

in cells in various processes of ion and organic compound uptake. The functional relation of H^+-pumps to the growth processes and plant productivity is also of great importance (Babayev et al., 2011).

The proton pumps exist in cells of all bio-organisms. According to Mitchell a redox chain, performing active transport of H^+, i.e. the proton pump is in plasmatic membrane of plant cells. Later the proton pumps were observed in the plasmatic membranes of algae (Spanswick, 1974) and higher plants. In higher plants, it is called ATP synthesizing pump-generator. Proton pumps were found to participate in the energy transformation and accumulation in chloroplasts and mitochondria. The active transport of H^+-ions was established to play an important role in ion absorption through roots (Pitman, 1970), functioning of bundle sheath apparatus (Raschke, 1971) and ion exchange in reserve tissues. Withdrawal of the H^+-ions from cytoplasm and entry of cations into the cell support cell trophism (Polevoy, Salamatova, 1979).

As there are no reports on the role of H^+-pumps, CA, RBPC and NAD-MDH localized in root cells, the research on their function in the creation of the defense reactions has to be continued (Liu et al., 2015).

MATERIALS AND METHODS

Plant material and growth conditions. Leaves and developing grains of wheat genotypes with contrasting productivity, drought tolerance, architectonics and other characteristic features have been used as the research objects. Durum wheat (*Triticum durum* Desf.) genotypes such as Barakatli 95 (drought-tolerant, high-productive), Garagylchyg 2 (drought-sensitive, high-productive) and Gyrmyzy bughda (drought-tolerant, low-productive) were used in the experiments. Wheat plants that are not exposed to stress factors were used as a control. Wheat plants were grown in soil, in a climatic chamber. The plants were cultivated at 23-25ºC, under fluorescent lamps LB-40 at 10-15 klx, at 12-h photoperiod, 60-70% humidity, which corresponds to the energy intensity of about 55-65 W/m^2. On the 7th day, the seedlings were placed into 50 ml weighing bottles. Weighing bottles were filled with the test solution, the volume of which during the experiment was kept constant by adding distilled water. Wheat seeds were grouped according to the morphological structure of embryo (Shevchenko, 1974). The leaves and roots of 3-10-day-old wheat plants were taken for the analysis.

Determination of gas exchange parameters: Parameters of gas exchange – rate of photosynthesis (P_n, μmol CO_2 $m^{-2}s^{-1}$), stomatal conductance (g_s, mol CO_2 $m^{-2}s^{-1}$), CO_2 concentration in intercellular space (C_i, μmol CO_2 mol^{-1}), transpiration rate (T_r, mmol CO_2 $m^{-2}s^{-1}$) were measured in leaves during the flowering phase at 10^{00}-12^{00}, using LI 6400 XT Portable Photosynthesis System (LI-COR 6400 Biosciences, USA). Plant leaves were placed into the 6 cm^2 chamber and after a short-term equilibrium, gas-exchange parameters were measured.

Enzyme extracts and subcellular fractions. Roots of the studied genotypes were washed in distilled water and dried. To perform homogenization 100 mM Tris-HCl buffer (pH 8.0) containing 1 mM EDTA, 5 mM DTT, 10 mM $MgCl_2$, 5% glycerol and 1% PVP (1:7 ratio, w/v; 4ºC) was used. After 2 minutes the obtained homogenate was filtered through 2 layers of a capron cloth and the filtrate was centrifuged for 5 min at 200 g to precipitate cell debris. The supernatant was centrifuged for 10 min at 1000 g. The resulting precipitate and supernatant obtained after centrifugation consist of chloroplast and cytosolic fractions, respectively. Each subcellular fraction was kept in the homogenization medium containing 0.5% Triton X-100 for an hour and formed enzyme extracts were used for further studies. The resulting supernatant was used to measure the activity of the enzymes.

Determination of CA activity. CA activity was measured using an electrometric method based on the releasing H^+-ions in the $CO_2 + H_2O \rightarrow H^+ + HCO_3^-$ reaction (Wilbur, Anderson, 1948). The reaction was carried out in 0.05 M Tris-HCl buffer (pH 8.1) at 2-4ºC by adding 10-200 μl of the enzyme preparation. The reaction started by adding 3 ml of the saturated solution of CO_2. The final volume of the reaction mixture was 20 ml. The changes in pH were measured with pH meter (universal ionomer ЭВ-74) and XY-RECORDER ENDIM 620.02.

The enzyme activity was estimated in standard units according to the formula (Rickli et al., 1964).

$$U=10\cdot(T_0/T-1)$$

where, T_0 is non-enzymatic reaction (control) time, T - enzymatic reaction time, U - the enzyme activity in conventional units.

The saturated solution of CO_2 was prepared in bidistilled water at 25°C and 1 atm pressure. The concentration of CO_2 was determined using the reverse titration with HCl in the presence of $Ba(OH)_2$.

Determination of RBPC activity. RBPC activity was determined spectrophotometrically, based on the quantitative assay of 3-phosphoglycerate kinase (3-PGK) and glyceraldehyde-3-phosphate dehydrogenase amounts (Romanova, 1980). RBPC activity was followed at 30 °C as the decrease of absorbance at 340 nm resulting from the oxidation of NADH to NAD (Ultrospec 3300, Amersham BioSci.). The reaction medium consisted of 0.05 M Tris-HCl buffer (pH 7.8) containing 0.05 M $NaHCO_3$, 0.01 M $MgCl_2$, 0.05 M DTT, 0.01 M ATP, 0.25 mM NADH, 0.3 mM RBP, 10 E 3-PGK, 10 E GAPDH. The reaction was initiated by adding 10 µl enzyme extract. All components except NADH were included in the control variant.

Determination of NAD-malate dehydrogenase (NAD-MDH) activity. NAD-MDH (EC 1.1.1.37) activity was determined spectrophotometrically (Scheibe, Stitt, 1988). 100 mM Tris-HCl (pH 8,0) buffer consisting of 10 mM oxaloacetate (OAA), 10 mg/ml bovine serum albumin (BSA), 10 mM $MgCl_2$, 12 mM NADH and 5-10 µl enzyme preparation were used as the assay mixture. The reaction was initiated by adding the substrate (10 mM OAA) to the reaction medium. 1ml cuvettes were used for the measurements. The enzyme activity determination was based on the decrease of optical density at 340 nm, in 1 min period.

The enzyme activity was estimated in standard units according to the formula

$$A=\Delta OD\cdot V/\varepsilon\cdot b\cdot\tau$$

A - activity in standard units, ΔOD – changes in optical density for 1 min, V - volume of the reaction medium (ml), τ - time spent for the reaction proceeding, ε - millimolar extinction coefficient, which equals to 6.22 $mM\cdot cm^{-1}$ for NAD^+ and NADH (coenzymes of NAD-MDH) at 340 nm wavelength, b-is a volume of the enzymatic extract added to the reaction medium (µl).

Determination of kinetic parameters. Kinetic parameters of the purified enzyme preparations were determined. The standard assay system consisted of 0.05 M Tris-HCl buffer (pH 8.1) and substrate CO_2. The K_m values and the maximal rates for the substrates were obtained from at least three experiments after the transformation of the data from the Lineweaver-Burk plots.

Determination of H^+-pump activity. The acidity of the nutrient solution was determined using the glass pH electrode (pH meter ”pH-340”) (Glass et al., 1981). Wheat seedlings were grown in a weak salt solution, then KCl solution was added for the stimulation of H^+-pump activity and kinetics of released H^+ was studied. After the lag period, kinetic profile became linear. The slope of the line is used as a measurement unit for the proton pump activity.

Total protein determination. Total soluble protein was measured by the method of Sedmak (Sedmak, Grossberg, 1977). Bovine serum albumin (BSA) was used as a standard.

Statistical analysis. The experimental results were processed statistically. Three different samples were taken for each treatment and they were analyzed twice.

RESULTS AND DISCUSSION

Affecting higher plants, salinity (Munns et al., 2006) and soil drought (Chaves, Oliveria, 2004) inhibit energetic processes such as photosynthesis and respiration. To study the effect of both stress factors on plants, first of all, we determined gas-exchange parameters (Table 1). As seen in the table, the change patterns of gas-exchange parameters are not similar under soil drought and salinity during the flowering phase. The increase in C_i during stress shows that when CO_2 diffusion through stomata is obstructed, the plant tries to provide a substrate for photosynthesis at the expense of the activation of the carbon concentrating mechanism (CCM). An increase in C_i observed in some cases under water stress can be attributed to the increase in resistance of mesophyll cells against CO_2.

Table 1. Effect of drought and salinity on gas-exchange parameters of wheat flag leaves during the flowering phase:

Varieties	Variants	P_n	g_s	C_i	T_r
		Drought			
Barakatli 95	C	16.3±0.72	0.43±0.021	309.0±8.0	6.89±0.42
	D	12.1±0.58	0.30±0.018	296.0±6.5	7.04±0.32
Garagylchyg 2	C	15.09±0.84	0.501±0.030	275.0±8.4	7.08±0.46
	D	11.4±0.53	0.299±0.015	300.0±6.9	4.90±0.51
Gyrmyzy bughda	C	12.3±0.72	0.399±0.034	323.0±7.2	7.34±0.27
	D	8.9±0.42	0.218±0.026	310.0±4.4	5.55±0.22
		Salinity			
Barakatli 95	C	19.5±1,37	0.79±0.063	245.0±7.22	3.34±0.20
	S	15.3±0.77	0.44±0.035	269.0±6.22	3.14±0.19
Garagylchyg 2	C	18.2±0.91	0.71±0.089	268.0±4.8	3.99±0.30
	S	14.8±0,86	0.59±0.042	299.0±5.3	3.36±0.33
Gyrmyzy bughda	C	15.6±0.98	0.67±0.068	301.0±3.5	2.90±0.26
	S	11.5±0.79	0.51±0.049	314.0±4.8	1.68±0.14

Note: P_n- µmol $CO_2 \cdot m^{-2}.s^{-1}$; g_s-mol $H_2O.m^{-2} \cdot s^{-1}$; C_i-µmol $CO_2 \cdot mol^{-1}$; T_r-mmol $H_2O \cdot m^{-2} \cdot s^{-1}$

Plants are known to respond to drought at the level of gas-exchange parameters, enzymatic activities, synthesized intermediates, etc. Therefore, the study of dynamics of the changes in intermediate contents is of great importance in the research of the salinity effect on the plant organism. It was found that the amount of proline, which is one of the representatives of intermediates 70% increased during the flowering phase compared with the control variant. Whereas, the amount of malone dialdehyde (MDA), which is the product of lipid peroxidation, increased by 40%.

The same tendency was observed in the pigment contents. Thus, a decline in the chlorophyll a/b ratio was accompanied by a significant increase in the carotenoid (Car) content. (Rampino et al., 2006)

Morpho-physiological properties of seed embryo allow evaluating growth and development of genotypes, their productivity and early maturation. Shevchenko divided wheat seeds into 6 bio groups based on the morphological structure of the embryo and on the grain yield. To find functional indices of grain yield, kinetics of active H^+-flows, which is the functional parameter of the productivity of wheat seeds, was studied in the root system of 3-9 day old seedlings of autumn wheat (Shevchenko, 1974).

Moreover, the dynamics in the activities of carbon metabolism enzymes, such as CA, NAD-MDH was studied in roots at increasing NaCl concentrations. It is known that abiotic factors (drought, salinity, etc.) effect on metabolic processes first of all, in root and leaf level and then adaptive changes occur at other levels.

As seen in Table 2, the strongest acidification (ΔH^+_{max}) among bio groups of Barakatli 95 occurred in the II bio group (ΔH^+_{max}= $3.8x10^{-4}$ µequiv/h). The highest ΔH^+_{max} values were detected in the I and V bio groups – (ΔH^+_{max}= 22.7 and $35.0x10^{-4}$ µequiv/h) and the lowest ΔH^+_{max} (ΔH^+_{max}=$2.33x10^{-4}$ µequiv/h) was observed in the I bio group. The same changes of ΔH^+_{max} occurred in the III and IV bio groups. Accordingly, the highest seedling was detected in the II bio group (12.8 cm). The highest rate of development was also observed in this group (2.98 cm per day). Barakatli 95 showed a mild degree of salt tolerance.

As seen in Table 2, the strongest acidification (ΔH^+_{max}) among bio groups of Garagylchyg 2 was observed in the II bio group (ΔH^+_{max}= $2.5x10^{-4}$ µequiv/h). The highest ΔH^+_{max} values were detected in the I and V bio groups – (ΔH^+_{max}= $2.7x10^{-4}$ µequiv/h) and the lowest ΔH^+_{max} (ΔH^+_{max}=$1.9x10^{-4}$ µequiv/h) was observed in the III bio group.

The Garagylchyg 2 variety, which is also productive, was practically intolerant: Root system H^+-pumps could not recover active H^+-outflow during two days. Although the plants did not perish during this period, the inhibition of the work of H^+-pumps may be a symptomatic sign indicating a weak resistance of this variety against salinity.

Table 2. Average weight of seeds (δ-standard error) in each bio group of three winter wheat varieties with different productivity (bio group volume is designated in %), rate of leaf growth (V_l, cm/day), maximal length of leaf (L_l, cm) and root (L_r, cm) and their ratio (L_l/L_r).

Varieties		Bio groups				
		I	II	III	IV	V
Barakatli 95	Weight	30.2±1.8	47.0±1.1	38.2±1.2	-	39.3±1.3
	%	6.7	40.0	20.0	0.5	33.0
	ΔH^+	22.7	3.8	3.5	3.5	35.0
	V_l cm/day	2.0	2.73	2.33	2.38	1.73
	L_l (cm)	10.6	12.8	10.2	11.1	8.6
	L_r (cm)	3.0	4.3	4.0	4.2	4.0
	L_l/L_r	2.53	2.98	2.55	2.64	2.15
Garagylchyg 2	Weight	22.0±2.7	43.0±1.0	32.0±1.1	-	44.0±1.3
	%	7.4	41.3	18.1	2.0	31.2
	ΔH^+	2.7	2.5	1.9	2.4	2.7
	V_l cm/day	1.68	1.4	1.8	0.55	2.2
	L_l (cm)	9.4	6.4	8.4	7.0	11.5
	L_r (cm)	4.5	4.2	4.4	4.2	4.3
	L_l/L_r	2.09	1.52	1.91	1.67	2.67
Gyrmyzy bughda	Weight	52.1±1.7	57.1±1.4	54.4±3.0	55.7±4.5	59.0±1.4
	%	32.8	21.2	15.1	13.5	17.4
	ΔH^+	2.1	2.0	2.0	1.9	2.0
	V_l cm/day	2.38	2.75	2.38	2.20	2.56
	L_l (cm)	11.2	10.5	12.9	12.5	14.1
	L_r (cm)	3.9	4.1	4.3	4.3	4.5
	L_l/L_r	2.87	2.56	3.0	2.91	3.13

Note: The values of the maximum acidification ΔH^+ (μequiv/h) created by the seedlings of each bio group are given for comparison. Estimated values of the average weight of seeds in the population are 41.42 mg for Barakatli 95, 38.9 mg for Garagylchyg 2 and 55.2 mg for Gyrmyzy bughda.

The variety with the lowest productivity - Gyrmyzy bughda appeared to be the most tolerant to salinity. The maximum rate of the proton release (ΔH^+_{max}) in the root systems of all bio groups of the Gyrmyzy bughda genotypes was similar (ΔH^+_{max} = 1.9-2.1 x 10^{-4} μequiv/h).

Table 2 shows that the bio groups of low productive varieties did not differ in seed weight and the rate of the active H^+-outflow though the seed weight is 32% higher in comparison with productive varieties. On the other hand, the I group of the high-productive variety, which was considered as a non-productive according to the Shevchenko method, has the lowest weight. But the II bio group as the most productive has the highest weight. The I bio group of the Garagylchyg 2 variety did not concede the II bio group in the weight of seeds.

Thus, according to the obtained data we conclude that the seed endosperm size does not necessarily correlate with the power of the H^+-pump work and on the contrary in high productive varieties with the high H^+-outflow, the weight of seeds is lower than in the low-productive Gyrmyzy bughda.

As there are no differences in root systems of the genotypes, the degree of the acidification of an environment allows evaluating the power of H^+-pumps. Genotypes can be lined according to the acidification degree of H^+-pumps as follows: the most productive Barakatli 95 variety, Garagylchyg 2 ranked second and low productive Gyrmyzy bughda. Regarding the growth rate of genotypes, low productive Gyrmyzy bughda and high productive Barakatli had almost the same rates of the development of leaves, which was higher compared with that of Garagylchyg 2 (Table 2). Salinization of sowing lands is one of the modern problems. When salt concentration increases in the soil, ions, entered into plant cells, affect biochemical mechanisms of metabolic processes, which results in a decline of the productivity of agricultural plants (Rampino et al., 2006). Therefore, the study and use of new approaches are needed for the analysis of plant salt tolerance.

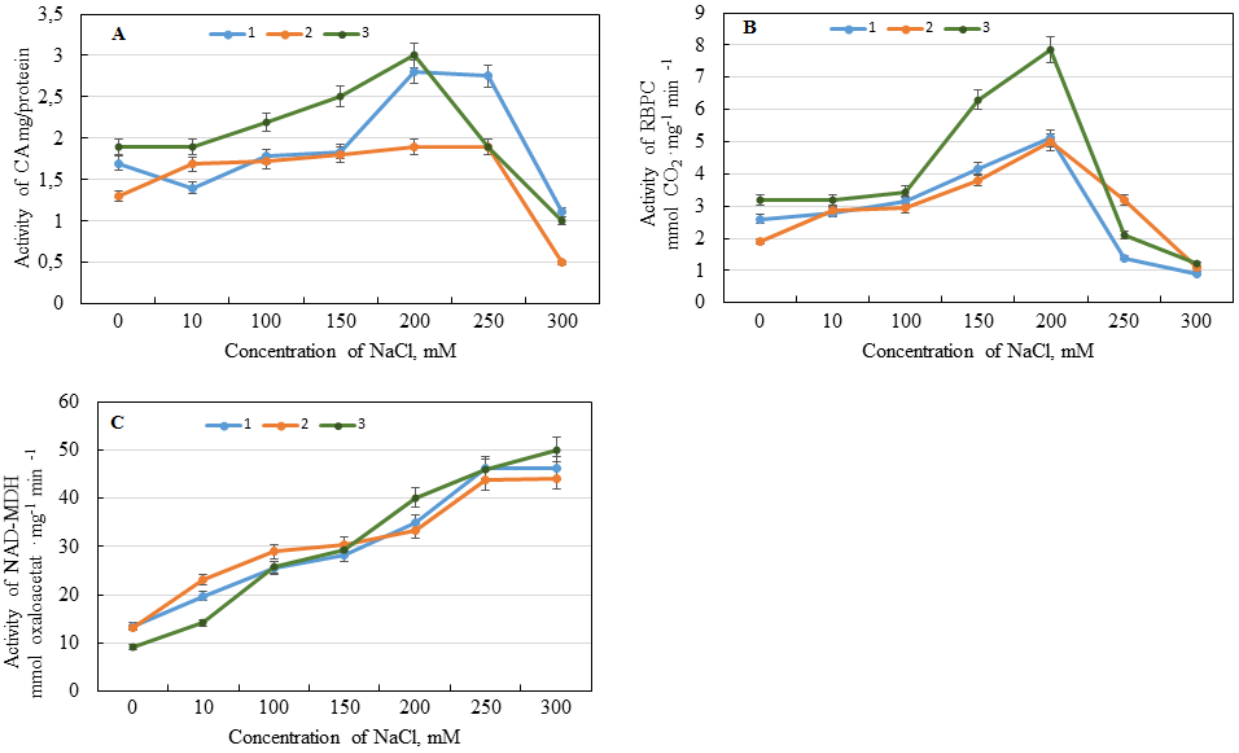

Fig. 1. The dynamics of the activities of carbon metabolism enzymes at high NaCl concentrations in leaves of different wheat genotypes. Designation: A-CA, B-RBPC, C-NAD-MDH; 1- Barakatli 95 (1); 2-Garagylchyg 2 (2); 3-Gyrmyzy bughda (3).

The study of the effect of salt stress on the activity of enzymes involved in the CO_2 metabolism in higher plants and on the activity of H^+-pumps in root cells is of great importance.

As seen in figure 1 the activities of CA (Fig. 1A), and RBPC (Fig. 1B) have changed similarly under various salt concentrations in leaves of the wheat genotypes. NAD-MDH activity was quite high at 300 mM NaCl (Fig. 1C).

Regulation of the activities of carbon metabolism enzymes is especially important. Due to stomata closure under salinity and soil drought, the amount of carbon dioxide absorbed from the atmosphere declines sharply causing disorders in the Calvin-Benson reactions and attenuation of the photosynthesis rate. To cope with CO_2 deficiency plants evolved Carbon Concentrating Mechanism. In the reaction catalyzed by Carbonic anhydrase, CO_2 is converted into bicarbonate ions (HCO^{3-}), which are transported to chloroplasts of plant cells. An increase in CA and NAD-MDH activities can stimulate activities of H^+-ATPases and thereby express adaptive property. It is assumed that NAD-MDH converts the rest of CO_2 formed during glycolysis and respiration in the root system into C_4 acids, such as malate and aspartate. Then, as a result of the decarboxylation of these acids, photosynthesis proceeds normally and plants can survive under stress.

It is assumed that CA localized in root cells participates in the utilization of CO_2 formed as a result of respiration. H^+ ions, which are the products of the hydration reaction catalyzed by CA, are output to the environment surrounding the roots by H^+-pumps. As plants have various H^+-ATP-ase activities, they differ in the acidity indices of the environment surrounding the roots, which leads to the differences in the levels of mineral nutrition.

To solve the mentioned problems, the study was performed at the increasing concentrations of salt and the obtained data are presented in Table 3. As seen in the table, at 10-150 mM NaCl, the rate of the proton release (ΔV_H^+) increased, whereas activities of CA, RBPC and NAD-MDH increased at 10-200 mM NaCl.

Table 3. The effect of various NaCl concentrations on the activities of CA, RBPC, NAD-MDH and H^+-pumps in wheat leaves and roots.

Genotypes			NaCl concentration, mM						
	Enzymes	Organs	C	10	100	150	200	250	300
Barakatli 95	CA	leaf	1.7	1.4	1.78	1.84	2.8	2.75	1.11
		root	1.8	1.6	1.65	1.65	2.5	2.53	1.0
	RBPC	leaf	2.6	2.8	3.16	4.16	5.1	1.39	0.91
		root	1.9	2.0	3.1	3.9	4.2	0.99	0.001
	NAD-MDH	leaf	13.6	19.7	25.6	28.2	34.8	46.3	46.2
		root	4.1	7.0	19.0	18.3	21.1	34.0	42.0
	$\Delta H \cdot 10^{-4}$ µequiv/h	root	0.2	0.14	0.31	0.98	0.8	0.16	0.01
Garagylchyg 2	CA	leaf	1.3	1.69	1.72	1.8	1.9	1.9	0.5
		root	0.7	1.2	1.65	1.65	1.65	1.11	0.4
	RBPC	leaf	1.9	2.85	2.93	3.8	4.98	3.2	1.09
		root	1.9	2.0	2.5	3.5	4.5	3.01	0.6
	NAD-MDH	leaf	13.2	23.1	28.9	30.5	33.3	43.9	44.0
		root	6.9	10.8	21.3	22.0	26.6	37.1	41.0
	$\Delta H \cdot 10^{-4}$ µequiv/h	root	0.2	0.23	0.47	0.92	0.49	0.12	0.01
Gyrmyzy bughda	CA	leaf	1.9	1.9	2.2	2.5	3.0	1.9	1.0
		root	1.8	2.1	2.5	3.0	3.0	1.43	0.6
	RBPK	leaf	3.2	3.2	3.44	6.3	7.85	2.1	1.2
		root	1.2	2.4	3.1	4.9	5.7	2.0	0.6
	NAD-MDH	leaf	9.2	14.2	25.7	29.3	40.1	45.9	50.0
		root	4.3	9.8	18.9	23.3	28.4	40.0	40.2
	$\Delta H \cdot 10^{-4}$ µequiv/h	root	0.3	0.24	0.49	0.88	0.51	0.15	0.01

Note: In the Barakatli 95, Garagylchyg 2 and Gyrmyzy bughda varieties seedlings of the II, V and II bio groups were used, respectively. $\Delta V_{H^+}=\Delta H \cdot 10^{-4}$ µequiv/h

CA and RBPC activities decreased and NAD-MDH activity increased, at 200-300 mM concentrations of NaCl. It should be noted that NAD-MDH activity increased even at 300 mM concentration of NaCl. The mentioned changes were more pronounced in the Gyrmyzy bughda variety.

CA activity decreased gradually after 200-300 mM NaCl in wheat leaves. Whereas, in cells of the root system the activities of this enzyme and H^+-pumps remained unchanged until the end of vegetation. Considering the above mentioned we can conclude that acting in concert, H^+-pumps and CA localized in root cells play a crucial role in the creation of tolerance against stress in higher plants.

Thus, CA loses activity under stress and participates in the utilization of CO_2 formed during the respiration of root cells. The second product of the reaction catalyzed by CA, H^+ ions participate in providing normal proceeding of the ion exchange in cells of the root system.

It should be noted that the increase of the plant productivity depends on the level of the physiological processes requiring high energy and harmonic functions of above-ground and underground parts of the plant. Thus, there is a positive correlation between the activity of H^+-pumps in the root system and activities of the enzymes involved in the carbon metabolism. Therefore, energy formed in the membranes of plant root cells by H^+-pumps is expended in various processes such as uptake of ions and organic compounds (Birktoft et al., 1989).

CONCLUSION

The evaluation of the potential productivity of the winter wheat on the basis of morpho-physiological types of the seed embryos showed that bio groups of the intensive type-II and IV predominated in more productive varieties while in less productive varieties the I bio group predominated. Differential tolerance of different bio groups within the sort population may serve as the selection sign to improve salt-tolerance in seed farming.

Within sort population of seeds, energetic estimation of bio groups of different productivity allows determining maximal H^+ gradients and the

rate of the active H^+-outflow.

Recovery of the work of H^+-pumps was characteristic of the low-productive Gyrmyzy bughda and high productive Barakatli 95 under salinity.

It suggests that a part of CO_2 formed during respiration and sent to carboxylation centers is being used in synthesis processes. The rest of CO_2 is converted into H^+ and HCO_3^- by CA. Formed H^+ ions penetrate to the medium by means of H^+-pumps and cause its acidification, i.e. formation of the specific rhizosphere. Therefore, the medium pH measurement allows estimating H^+-pump and CA activities.

The increase in NaCl concentration to 200 mM caused an increase in ΔH^+, ΔV_L, activities of CA, RBPC and NAD-MDH activity decreased. This increase is more pronounced in tolerant and low-productive Gyrmyzy bughda and in tolerant and high-productive Barakatli 95 contrary to Garagylchyg 2, which is intolerant and productive variety.

At 200-300 mM NaCl concentrations, ΔH^+ and ΔV_L, as well as CA and RBPC activities decreased and NAD-MDH activity increased. It may be explained as follows: the increase in NAD-MDH activity caused the intensification of synthesis of C_4 -acids (malate, aspartate) and in spite of the closure of bundle sheath cells, Calvin cycle reactions were not disturbed. Because malate and aspartate provide Calvin cycle with CO_2 when its intake from atmosphere terminated and gas-exchange is attenuated.

Controversial issues concerning the localization of CA in the root system of higher plants were clarified and the presence of CA in the root system cells was confirmed. A positive correlation was detected between the activity of H^+-pumps realizing ion-exchange in root system cells and the activities of the enzymes CA and NAD-MDH, localized in roots and leaves.

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Duz stresi şəraitində buğda genotiplərinin kök hüceyrələrində bəzi karbon metabolizmi fermentlərinin və H^+-nasoslarının aktivliyi

H.Q. Babayev. U.Ə. Qurbanova. N.M. Quliyev

[1]AMEA Molekulyar Biologiya və Biotexnologiyalar İnstitutunun Karbonun fotosintetik assimilyasiyasının enzimologiyası laboratoriyası, Bakı, Azərbaycan

Bu məqalədə buğdanın məhsuldarlığına və quraqlığadavamlılığına görə fərqlənən Bərəkətli-95, Qaraqılçıq-2 və Qırmızı buğda genotiplərinin kök hüceyrələrində Karboanhidraza (KA), NAD-Malatdehidrogenaza (NAD-MDH). Ribulozo 1.5-bisfosfatkarboksilaza (RBFK) və H^+-nasoslarının bitkilərdə şoranlığın təsirinə qarşı adaptiv reaksiyaların yaranmasındakı rolu tədqiq olunmuşdur. KA fermentinin bitkilərin kök sistemlərində lokalizasiyası ilə əlaqədar mübahisəli məsələlərə aydınlıq gətirilmiş və mümkün fizioloji-biokimyəvi funksiyaları haqqında mülahizələr söylənilmişdir. Müəyyən olunmuşdur ki. buğda genotiplərinin kök hüceyrələrində KA, NAD-MDH, RBFK və H^+-nasosları uzlaşdırılmış fəaliyyət göstərməklə ali bitkilərdə stresin təsirinə qarşı davamlılığın yaranmasında iştirak edirlər.

Açar sözlər: *Buğda, kök hüceyrələri, duz stresi, KA, NAD-MDH, RBFK, H^+-nasosları, davamlılıq*